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STUDIES ON PLANT CANCERS—II

THE BEHAVIOR OF CROWN GALL ON THE RUBBER PLANT (*FICUS ELASTICA*)[1]

(WITH PLATES 1 AND 2)

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Toumey (1900) in studying the effects of crown gall on the host pointed out the destructiveness of this disease on deciduous trees. He gave an adequate picture of the developmental stages in the growth of the crown gall tissue on the almond. He contends that the period of growth of the crown gall is definite and usually extends over the growing season; after which time the gall dies, falls out, leaving an open wound. In the following spring a new crown gall is formed on the margin of the old wound which in turn dies and increases the area of the lesion, so that it weakens the tree and causes it to break off in a wind, thus killing it. It appears from Toumey's study that death is the result of a mechanical effect of the crown gall on the tissue of the host in no way similar to the toxic effects that the cancerous growth has on the animal or human being.

Hedgcock (1910[1]) in his field studies of the effect of crown gall on grape showed that the crown gall stunts the plant and that when the galls occur on the stem under the ground they commonly decay, killing the adjacent tissue and often killing the vine above the point of attack. Whether the decay is directly brought

[1] From the Cancer Research Laboratory, Montefiore Hospital, Dr. Isaac Levin, Chief. The first paper was published in Bull. Torrey Club 46: 447–452. *pls. 17, 18.* 1919.

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about by *Bacterium tumefaciens* Hedgcock does not state. He claims however with Toumey that the galls die annually. In a later study (1910[2]) of crown gall on the apple he maintains that the destructive effect of this disease is overrated.

Smith (1911–12) in his extensive studies on crown gall and its resemblance to animal cancer shows that the physiological effects of these tumors vary from species to species and also within the species and are generally less pronounced and speedy than one might expect. He holds that it is difficult to show conclusively that the substances produced in the tumor by the parasite are absorbed and act as slow poisons. This is especially difficult in view of the fact that the galls are often soaked by rains and become infected with other parasitic and saprophytic organisms.

Levin and Levine (1918–20) in a report on the malignancy of the crown gall and its analogy to human cancer pointed out that a number of the phenomena in both diseases are analogous. They contend that the neoplasms in plants produced by *Bacterium tumefaciens* are sometimes benign though some are true malignant growths. The latter generally dwarf the plant so affected and cause necrosis of the tissue above and below the gall.

These studies and those of Smith's and other workers were carried out in annuals, biennials or deciduous trees in which the period of growth of the host as well as the crown gall is normally interrupted. The difficulty in determining whether toxins are present in such cases is made more difficult by the intervention of natural death, caused by changes in temperature and its concomitant factors, and second, by the occurrence of infections caused by fungi and even insect grubs, the eggs of which are deposited in the soft tissue of the young crown gall.

The purpose of this report is to bring forward further evidence on the malignancy of the crown gall experimentally induced on mature evergreen perennials such as the common rubber tree, *Ficus elastica*. In such plants where the growth is rather active all the year round, when kept under uniform, green house conditions, the effect of the crown gall organism and the neoplastic growth on the host can be kept under observation for an extended period. Drenching rains and destructive insects are

avoided and very often other parasitic and saprophytic fungi. In this way and in such plants as *Ficus elastica* it is possible to show definitely whether and in what degree the crown gall has an injurious effect upon the adjacent normal tissue of the host. It must be remembered however that while transportation of the materials elaborated by the cancer cells of the animal is in some degree limited, this is much more the case in plants.

I have found some evidence of injurious effects spreading from a gall upwards and finally killing the stem above the point of inoculation. This was the result in every case (10 branches) with two exceptions. In the first the signs of death are only now, 14 months after inoculation were made, making their appearance. In the other case described below, the stem, it appears, was cut off for examination too soon.

In no case was there any evidence that the death of the stem above the gall was due to the obstruction of the sap flow or water supply. Toumey's results do not suggest the possibility of any such direct mechanical disturbance on the part of the gall. I will describe briefly a number of cases observed.

Material and Observations.—Through the courtesy of Dr. S. Wachsmann, director of the Montefiore Hospital, a number of rubber trees (*Ficus elastica*) were placed at my disposal. These plants were growing in large boxes and were kept indoors during the winter months in a basement room well lighted and ventilated. In the summer these plants were moved out on the campus of the hospital. These plants make almost as much growth during the winter as they do during the summer. Various parts of these plants were inoculated with *Bacterium tumefaciens,* labeled and then examined from time to time. It was found that within a month indications of a crown gall made their appearance in the part of the plant inoculated.

Figure 1 represents one of the trees during the month of January used in this study. The terminal buds are opening and the moderately green glistening apical leaves show evidence of an active condition of growth. The plant shown in figure 1 with five others of equal size were inoculated on July 28, 1919, by pricking the tissue with a steel needle that had been previously

dipped into a culture of *Bacterium tumefaciens.* As few as five pricks of the needles with the crown gall organism were found to be sufficient to produce a visible neoplasm in a month's time. A careful scrutiny of this picture reveals a number of galls at the internodes of several branches (Fig. 1, *a, b*) on the mid-vein of an old leaf (Fig. 1, *c*) and on one of the main branches (Fig. 1, *d*). Where the needle perforated the tissue a crown gall was formed on both sides of the stem or the leaf. No less active were the growths that were produced on the trunk of the tree (Fig. 1, *d*). The galls formed are of the characteristic type described by Toumey, Smith and others. They are always firm, yellowish in color and covered with brownish patches when young and become dark brown in color and of a woody consistency with age, as we shall see below.

The crown gall, at this stage, as far as can be seen, has no specially injurious effect upon the host. The terminal buds of the plant are actively growing and there appears to be no signs of dwarfing of the branches, nor any indication of fascination of the internodes above the region of inoculation such as those reported by Smith, and Levin and Levine for *Geranium, Ricinus,* etc.

Figure 2 represents a branch from another rubber tree which had been inoculated seven months previously on the second internode. The crown gall has grown extensively, covering one half of the circumference of the stem. The surface is dark brown in color, highly convoluted, indicating a number of centers of peripheral growth. The mass is hard and some parts of the surface appear to be dead. The branch however has grown considerably as shown by the number of internodes above the crown gall. (See Figs. 2 and 3.) In June, 1919, it was noted that the terminal bud was small and dark green in color, and showed no signs of growth. This was true of a number of other branches which had been inoculated for the same length of time. The control branches that were similarly treated with a sterile needle had long greenish buds, many of which were opening. This condition suggested at once the possibility of mechanical interference of the crown gall with the water supply of the plant due to partial destruction and possible occlusion of the fibrovascular bundles,

but cross and longitudinal sections of this gall made much later showed this assumption to be incorrect as is further described below. It is obvious at once however that there is some other cause of death than the cutting of the water supply, since in that case, the dying would progress from the tip downward.

Twelve months after inoculation. Figure 3 represents the same branch shown in figure 2 on December, 1919, approximately 12 months after the inoculation had been made. The crown gall has almost girdled the stem encircling 7/8 of the stem's circumference. The leaves above the crown gall have turned black and fallen off while those below are turning a yellowish brown. The major portion of the stem above the gall is dead, the injury progressing from the gall upward so that at the time the photograph was made the top of the stem (Fig. 3) was still green and showed indications of being alive. A cross section through the middle of this crown gall appears in figure 4 and shows that the crown gall tissue has become fully differentiated and thus further supports the contention of Toumey and Hedgcock that the crown gall growths are annual and Levin and Levine's views that these growths are unlike animal cancers in that they are limited in growth and become differentiated. The wood fibers and parenchymatous cells of which the crown gall is composed are dark brown on the interior of the gall as they are on the surface. The vascular elements are distorted and nodular on the periphery of the tumor where their antecedents were undoubtedly centers of rapid cell division before they became differentiated and old.

Approximately one half of the original cylinder made by the fibrovascular bundles is destroyed and replaced by crown gall tissue. The tissue in the center of the crown gall is dark in color, watery and is apparently disintegrating. The remaining half of the wood cylinder appears to be undistributed and undoubtedly is mechanically fit to carry sap, as evidenced by the still turgid condition of the top of the branch as shown in figure 5. This figure represents a longitudinal section of the upper part of the stem including the upper part of the crown gall. There appears to be only a partial destruction of the wood fibers in the region of the stem occupied by the lesion as seen in the

cross section to the left of the figure. The wood and pith are apparently normal structurally though physiologically dead.

A later state in the necrosis following the inoculation of *Bacterium tumefaciens* is shown in figure 6 photographed eleven months afterward. Here again the inoculation was made at one side of the stem in the third internode with a needle dipped into an emulsion of the crown gall organism. In this late stage the growth does not completely girdle the stem, yet two months previously the leaves fell off and the stem became discolored and finally died. The crown gall and the stem above the gall also died. A short portion of the stem immediately below the gall at the time of the photograph was rapidly undergoing similar changes. The gall in this case again shows all the characteristics of the typical crown galls referred to above. The outer surface is dry and woody and is markedly nodular. In a longitudinal section of this gall we find the region near the stem slightly moist, darker in appearance and invading the wood cylinder (Fig. 7). A large portion of the wood cylinder is intact and appears to be functional. There again, it appears as if death was caused by *Bacterium tumefaciens* or the crown gall cells rather than by the interference with the transportation system caused by the destruction on the invasion of the fibrovascular bundles.

Figure 8 represents a gall 12 months old which has caused no injury to the stem either above or below the gall. Growth is continuing normally. The inoculation was made in two opposite sides of the branch. The crown gall that appears in front on the stem "B" and "C" was produced by inoculating an axillary bud region. The lower gall, "A," was obtained by inoculating an internodal space on the opposite sides of the stem. The lower growth which appears as two separate tumors on opposite sides of the stem consists of one continuous mass of tissue encircling one half of the circumference of the stem. The growth has a distinctly tubercular structure. It is dark brown in color, hard and dry, and apparently dead. The upper crown gall which is on the surface of the stem, as mentioned above, extends for a distance of nearly one half of the circumference of the stem also. To the left it developed into a more or less uniformly globular growth through which two branches have grown.

We may turn now to consider the cases in which no evidence of injurious effects of the gall in tissue above and below it in the stem have yet appeared. In all, I have observed two such cases as mentioned above against ten in which death of the region above and below the gall or both occurred.

It is natural to suspect in view of the statements of the authors quoted that the injurious effects I have observed may be due to the presence of some additional infection or to some special direct physical effect of the crown gall on the rubber tree. I am however convinced that this is not the case.

As in the case of *Bryophyllum* (Levine 1919), *Bacterium tumefaciens* does not cause the formation of embryomata when inoculated into *F. elastica* in a region where embryonic cells are to be expected. At the time this photograph was made, twelve months after inoculation, the upper gall was still active although parts of it were beginning to distintegrate. The stem above the gall appears as noted to be entirely unaffected and in good physiological condition. A cross section of the stem made at the level indicated by the line "AA" shows complete disorganization of more than one half of the wood cylinder. The remaining half is not unlike the apparently healthy portion of the wood shown in figure 4. A photograph of the cut end of the stem at the level of "BB" (Fig. 8) is shown in figure 9. Here little of the vascular cylinder appears to be invaded by the crown gall tissue. At this level the great mass of the crown gall seems to have developed from the cortical layer of the stem only and has not, at this time, affected the central cylinder.

The gall from which the branches "Y" and "Z" appear (Fig. 9) is unlike all other crown galls so far described in that almost its entire surface is smooth and not tubercular; it is covered with small brown corky patches. The lower left side of the gall in the picture shows the typical crown gall convolutions.

A section still higher up on the stem made at the level indicated by the line "CC" cuts through this smooth gall at a point near the origin of the branches "Y" and "Z" (see Fig. 10). An abundance of milk comes from the entire surface above the dark area of the crown gall shown in this figure. No invasion

of the central cylinder by the crown gall tissue appears. There is, however, a slight hyperplasia of the wood. The fan-shaped vascular elements in the gall seem to be running into the branches "Y" and "Z" from "X." The gall in this case may be compared to the so-called benign tumors (Levin and Levine, 1918). The character of the tissue of this neoplasm does not differ from that of a malignant crown gall. It seems obvious that the death of the crown gall is in general a result of merely mechanical conditions. The gall may be insufficiently supplied with food and water and dies because it fails to establish an adequate connection with the conducting system of the host. It is most likely that this is true of the almond crown gall described by Toumey.

Bacterium tumefaciens from stem and crown gall. The possibility that another organism as well as *Bacterium tumefaciens* is present and is responsible for the destruction of the stem apex as shown in figures 3, 5, 6 was tested in the following manner. Small pieces of the interior of the crown gall shown in figures 3 and 6 were carefully removed with a sterile knife and placed in tubes of beef agar. In two days the surfaces of the agar on which the inocula were resting became covered with a hyaline, whitish yellow colored schizomycete which in general appearance is not unlike that of *Bacterium tumefaciens.* Similar results were obtained by planting pieces of the stem from above the crown gall after being superficially sterilized by immersing in a weak formol solution. In all tubes the organisms were more or less alike in their superficial appearance. In several beef agar cultures the hyphae of a mold made their appearance. The presence of the mold we consider accidental contamination. Molds at any rate are not known to be parasitic on and to cause death of the rubber tree.

It appears from this that the organism is carried to parts removed from the gall but owing to its depauperate condition is unable to influence the production of a new growth.

The organism obtained in these cultures were inoculated into the tissue of young growing geranium plants and young shoots of the rubber trees. Crown galls appeared within two months after inoculation. The growths were much smaller than those

obtained by inoculating young geraniums and branches of a rubber tree with a known culture of *Bacterium tumefaciens.* This supports the contention that the bacteria in the distant parts of the stem bearing a crown gall are of a less virulent strain.

SUMMARY

1. *Bacterium tumefaciens* inoculated into the apical internode of the branches, into the leaves, or main stem of the rubber tree, *Ficus elastica,* stimulates the development of a neoplasm in the region of inoculation of a benign or malignant nature. The crown galls so formed, in this plant, are of two kinds, one in which growth is uniform and appears to be a swelling, the other is the characteristic convoluted type indicating a peripheral growth of isolated nodules.

2. The early stages in the development of the crown gall in *Ficus elastica* does not interfere with the life of the plant as a whole nor does it interfere with the growth of the inoculated branches.

3. The crown gall in *Ficus elastica* after a number of months of active growth becomes hard and dry and finally dies. This is associated with the differentiation of the tissue which converts the gall into a mass of parenchymatons cells and nodules of woody fibers. The central portion of the crown gall which generally lies near the woody cylinder disintegrates.

4. The invasion of the stem by the new growth does not destroy the entire conducting system of the stem, yet that portion of the stem above the gall dies as well as considerable portion of the stem below.

5. Cultures made from pieces of the crown gall and stem above the gall yield only a schizomycete which in appearance is not unlike *Bacterium tumefaciens* and which when inoculated into the stem of young geranium and rubber plants produce crown galls in the region of inoculation.

6. It is possible that the crown gall cells or the crown gall forming organisms are responsible for the progressive necrosis of the stem from the gall upward and downward. The death of the plant due to crown gall is at least suggestive of the death caused by malignant growths in animals.

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EXPLANATION OF PLATES 1 AND 2

Fig. 1. Represents the type of *Ficus elastica* used in these experiments. The galls in the various parts of the plant *a, b, c,* and *d* are the result of inoculating them with a culture of *Bacterium tumefaciens,* five months previously.

Fig. 2. Apical portion of a branch showing a large crown gall seven months after inoculation in the second internode. The gall does not seem to have interfered with the growth of the stem; several internodes having been added in the interim. (× ½.)

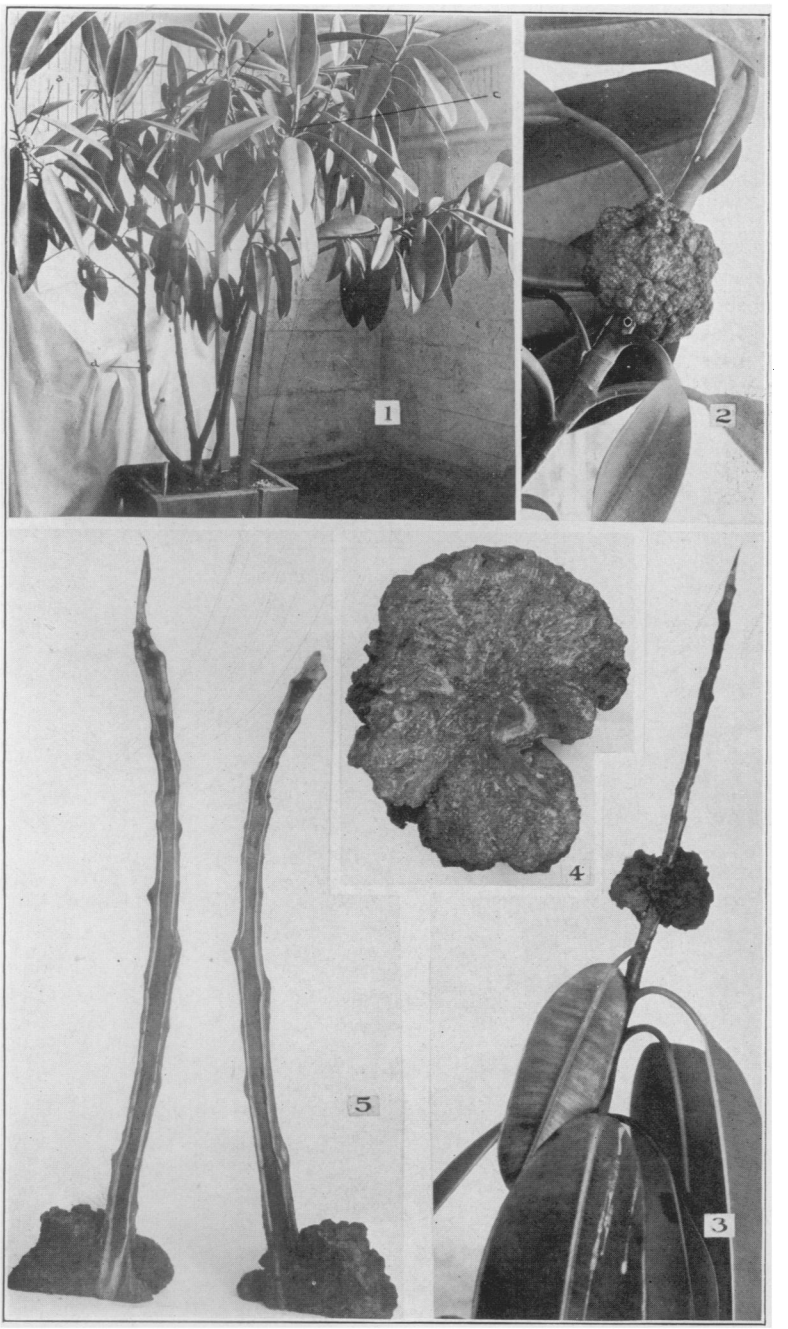
Fig. 3. Same branch twelve months after inoculation. The leaves above the gall have dropped off. The stem is discolored, dry, and dying progressively upward. The tip is still green and alive. The gall is hard, dry and dead. (× ¼.)

Fig. 4. Cross section of the stem through the gall shown in figure 3. The wood cylinder is only partially destroyed by the invading gall. The portion of the crown gall near the central cylinder is soft and disintegrating.

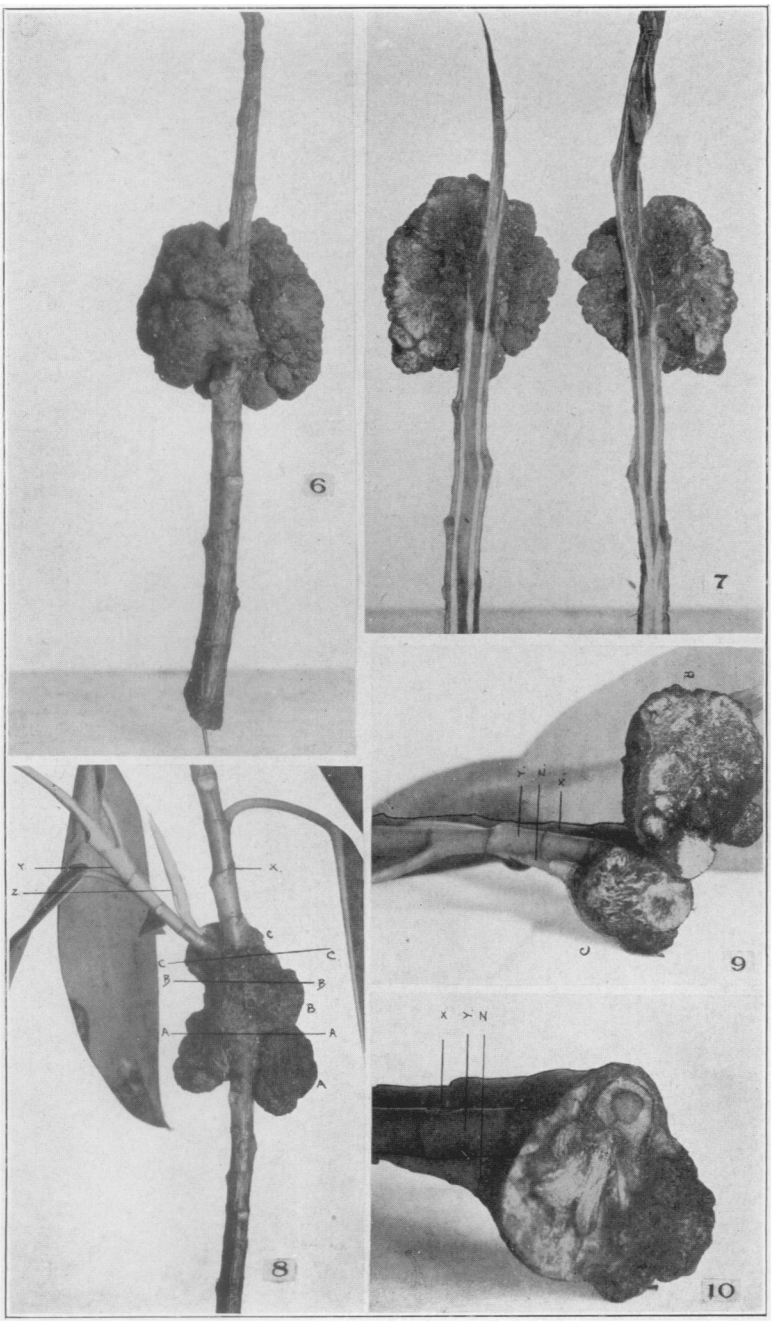
Fig. 5. Longitudinal section of the upper portion of the same stem. The portion near the gall is dry, brown and dead, while the apical internode and bud are still green and alive.

Fig. 6. A branch of *Ficus elastica* in which the gall and the stem above and below the gall is dead; the inoculation having been made twelve months previously.

Fig. 7. Longitudinal section showing invasion of the crown gall destroying a considerable portion of fibrovascular bundles. The invading portion of the gall is soft, spongy and disintegrating.



BACTERIUM TUMEFACIENS ON FICUS ELASTICA



BACTERIUM TUMEFACIENS ON FICUS ELASTICA

Fig. 8. A branch of *Ficus elastica* actively growing twelve months after having been inoculated with *Bacterium tumefaciens*. Two galls formed "A," and "B," "C," on opposite sides of the stem showing the smooth and nodular types of crown galls. Two branches are growing through the smooth crown gall.

Fig. 9. Cross section of stem between the upper and lower crown galls corresponding to level indicated by the line "BB" in figure 8. The gall to the left is of the smooth kind, being covered by corky patches.

Fig. 10. Cross section higher up on the stem corresponding to the level indicated by the line "CC" in figure 8. Shows large brown necrotic area and the undisturbed cylinder of the main stem "X" with bundles of fibers going to branches "Y" and "Z."